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Award Number: DAMD17-00-1-0024

TITLE: Relationships Between IGF-1, IGF-Binding Proteins and
         Diet in African American and Caucasian Men

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REPORT DATE: January 2002

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
                Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
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REPORT DOCUMENTATION PAGE	*Form Approved* *OMB No. 074-0188*

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE January 2002	3. REPORT TYPE AND DATES COVERED Annual (15 Dec 00 – 14 Dec 01)	
4. TITLE AND SUBTITLE Relationships Between IGF-1, IGF-Binding Proteins and Diet in African American and Caucasian Men		**5. FUNDING NUMBERS** DAMD17-00-1-0024	
6. AUTHOR(S) Tanya Agurs-Collins, Ph.D.			
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Howard University Cancer Center Washington, DC 20060 **E-Mail:** tagurs-collins@fac.howard.edu		**8. PERFORMING ORGANIZATION REPORT NUMBER**	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012		**10. SPONSORING / MONITORING AGENCY REPORT NUMBER**	

20020930 076

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited	**12b. DISTRIBUTION CODE**

13. ABSTRACT *(Maximum 200 Words)*

The study aims to determine racial differences between insulin-like growth factor-1 (IGF-1), insulin-like binding protein-2 (IGFBP-2), insulin-like binding protein-3 (IGFBP-3), prostate specific antigen (PSA), testosterone, body mass index (BMI), and diets high in calories, protein and fat. Specifically, the study objectives are to:

- define racial differences in serum levels of free and total IGF-1, IGFBP-2, IGFBP-3, and testosterone;
- define how diet and BMI impact serum levels of IGF-1, IGFBP-2, IGFBP-3, testosterone and PSA in African American and Caucasian men; and
- determine the associations between serum levels of free and total IGF-1, IGFBP-2, IGFBP-3, testosterone, PSA, BMI and specific nutrients.

The proposed study will help to explain the increased risk of prostate cancer for African American men and the role of specific nutrients in influencing IGF-1 and IGF-binding protein concentrations. This report covers primarily patient accrual activities during the first and second year of the project. These activities include finalizing the clinical protocol, hiring and training of study personnel, reviewing clinical questionnaires to determine study eligibility, and determination of stored frozen samples for use. A total of 544 men were recruited to participate in the study. Approximately 599 assays have been analyzed for IGF-1, IGFBP-2, and IGFBP-3. A total of 100 men were stratified and randomized for the telephone interview. Of this number, 25 men were interviewed to determine nutritional intake.

14. SUBJECT TERMS insulin-like growth factor-1 (IGF-1), insulin-like binding protein-2 (IGFBP-2) insulin-like binding protein-3 (IGFBP-3); prostate specific antigen (PSA); body mass index (BMI)			**15. NUMBER OF PAGES** 37
			16. PRICE CODE
17. SECURITY CLASSIFICATION OF REPORT Unclassified	**18. SECURITY CLASSIFICATION OF THIS PAGE** Unclassified	**19. SECURITY CLASSIFICATION OF ABSTRACT** Unclassified	**20. LIMITATION OF ABSTRACT** Unlimited

NSN 7540-01-280-5500

Standard Form 298 (Rev. 2-89)
Prescribed by ANSI Std. Z39-18
298-102

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Introduction

Presently, prostate cancer is the most common cancer in U.S. males. In 1999, the American Cancer Society estimates that 179,300 new cases will be diagnosed and approximately 37,000 men will die from metastatic prostate cancer (1). The incidence and mortality rates are even greater in African-American men than among other racial or ethnic populations in the world. Prostate cancer incidence rates are nearly two times higher for African-American men than for white men (2). The incidence and mortality rate for prostate cancer in the Washington, D.C. area is the highest in the world. Moreover, the rate of increase in prostate cancer occurs earlier for black males than white males (3). Evidence suggest that African Americans may be at higher risk since they consume diets higher in energy and fat and have made smaller changes in decreasing fat intake when compared to Caucasian men (4).

Insulin-like growth factor-1 (IGF-1) and IGF-binding proteins have been implicated in the carcinogenesis of breast, prostate and other hormone dependent cancers. Insulin-like growth factor-1 functions in an autocrine and paracrine manner to promote normal growth and malignant cellular proliferation (5-7). IGF-1 is produced by normal prostate cells (8) prostate cancer cells (9) and has mitogenic and antiapoptotic effects (10,11) on prostate epithelial cells (12). Several epidemiological studies have shown increased plasma levels of IGF-1 to be a strong risk factor for prostate cancer (13-15). Chan et al. (14) examined plasma levels of IGF-1 and IGFBP-3 in a prospective case-control study and found mean levels of IGF-1 to be significantly elevated among the prostate cases when compared to the controls. The relative risk was 4.3 (95% CI= 1.8-10.6) for men in the highest quartile of IGF-1 levels when compared with men in the lowest quartile. Higher plasma IGF-1 concentrations were associated with higher rates of malignancy in the prostate gland. Also, plasma levels of IGFBP-3 were inversely associated with risk after controlling for IGF-1 levels.

Another study (15) found a statistically significant positive association between serum levels of IGF-1 and risk of prostate cancer (OR=1.51; 95% CI=1.0-2.26 per 100 ng/ml increment). In this study serum levels of IGFBP-3 were not significantly associated with prostate cancer risk. However, Kanety et al. (16) found that patients with metastatic prostate cancer had significant reductions in both the absolute and relative amounts of IGFBP-3 and significantly higher serum IGFBP-2 concentrations when compared with the controls. The authors suggested that IGFBP's might be involved in growth modulation of prostate malignancy.

Several researchers have reported elevated serum IGFBP-2 concentrations (16-18) in patients with prostate cancer. It was suggested that elevations in serum IGFBP-2 concentrations might be unique to the carcinomatous condition (17). Ho et al. (18) suggested that IGFBP-2 might function as an IGF scavenger when the capacity of

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IGFBP-3 to bind IGF-1 in the serum is insufficient in patients with prostate cancer. Taken together, these studies strongly support a relationship between IGF-1, specific IGF-binding proteins and prostate cancer risk. To date, no published studies have examined racial difference in IGF-1 levels or systematically examined these associations in a healthy high risk screening population.

Prostate Specific Antigen (PSA), produced by the prostate epithelium, is elevated in patients with prostate cancer. Thus, PSA is considered a sensitive marker to monitor and detect disease. Studies show that PSA correlates with IGF-binding proteins. Ho et al. (18) found a positive correlation between serum levels of IGFBP-2 and PSA levels in patients with prostate cancer. The study results suggest that serum IGFBP-2 levels, like PSA, may reflect the tumor load in prostate cancer. Kanety et al. (16) also found that serum IGFBP-2 levels and its percentage of the total IGFBPs were highly positively correlated with serum PSA. In that study, a negative correlation was also found between IGFBP-3 and PSA. (16). These studies are consistent with findings in another study that showed IGFBP-2 elevated to a similar mean level when serum PSA was greater than 150 ug/l (17). It was suggested that the proteolysis effect of PSA on IGFBP potentiates the growth-promoting effects of IGF-1 on prostate cells. The researchers believe that PSA might serve to modulate IGF function within the reproductive system or in prostate cancer by altering IGF-IGFBP-3 interaction (17).

Researchers have examined various androgens as possible risk factors for prostate cancer. Ross et al (19) demonstrated that young African-American men had serum testosterone levels that were approximately 15% higher than their white counterparts. Research conducted by Erfurth et al. (20) showed that in a group of healthy men serum levels of IGF-1 increased with increasing free testosterone (p=0.005). In this study IGFBP-1 was significantly and positively correlated with free-testosterone and total testosterone.

Environmental factors, such as obesity and diet, have been shown to influence prostate cancer risk. Obesity has been shown to be associated with endocrine changes and is believed to be a risk factor for prostate cancer. Although the relationship between prostate cancer and obesity is somewhat inconsistent, two retrospective studies (21,22) and several prospective studies (23-26) have reported associations with body mass index (BMI) and prostate cancer risk. Andersson et al. (26) conducted a prospective study of 135,000 male construction workers who were followed for an average of 18 years. This study revealed a positive association of weight, height, BMI and lean body mass with risk of prostate cancer. Moreover, these anthropometric measures were more strongly associated with mortality. Obesity is also believed to be associated with IGF-1 levels. In a study of healthy males, free IGF-1 concentrations were higher in obese subjects than in normal controls (27). IGFBP-2 concentrations were also suppressed in the obese subjects. The researchers suggested that overnutrition and chronic hyperinsulinamia in obesity might alter the regulated growth response by insulin stimulation of IGF-1 production and suppression of hepatic IGFBP-1 and IGFBP-2 production, which may inhibit IGF-1 bioactivity.

Nutrition is a key regulator of IGF's and IGF-binding proteins (28) and prostate cancer risk. Specifically, energy restriction is associated with lower concentrations of IGF-1 (28,29) and a reduction in tumor growth, thus favoring cell apoptosis over cell proliferation (15). Isley et al. (30) showed diets deficient in protein and energy intake

decreased IGF-1 levels. In this study, changes in serum IGF-1 concentrations correlated significantly with mean daily nitrogen balance. Also, serum levels of IGFBP-2 and IGFBP-3 are inversely regulated by dietary protein and caloric intake as well as fasting (28). Investigators (31-33) have shown significant positive associations between total energy intake, dietary fat intake and prostrate cancer risk. These associations were more pronounced for cases with aggressive cancers (31,33). Andersson et al. (33) hypothesized that a high-energy, high fat, high-protein diet might influence prostate cancer risk mediated by IGF-1 concentrations. However, the relationships between IGF-1 and specific nutrients are not well understood, and those factors and the mechanisms of action requires further study.

Diet and obesity may play a significant role in understanding the relationships between serum IGF-1, IGFBP-2 and IGFBP-3 concentrations and prostate cancer risk. We believe serum levels of IGF-1, IGFBP-2 and IGFBP-3 may influence the etiology of prostate cancer and can serve as markers for this disease. Also, a low-fat, high-fiber diet has been shown to decrease circulating testosterone levels by altering male sex hormone metabolism (34,35). The proposed study can increase our understanding of the role of diet and obesity in modulating serum IGF-1 and IGF-binding proteins. Thus, reducing body weight/body fat may prevent or reduce prostate cancer risk. Understanding the associations between IGF-1, specific IGF-binding proteins, testosterone, PSA, BMI and diet in a healthy, screening population may help to better understand the etiology of this disease.

Hypotheses/Purpose

The purpose of this study is to examine racial differences in prostate cancer risk in a healthy high risk screening population of African American and Caucasian males. The associations between IGF-1, IGF-binding proteins (2&3), PSA testosterone, and BMI will be examined. Study hypotheses to be tested are:

The study aims to determine racial differences between IGF-1, IGFBP-2, IGFBP-3, PSA, testosterone, BMI, and diets high in calories, protein and fat. Specifically, the study objectives are to:
- define racial differences in serum levels of free and total IGF-1, IGFBP-2, IGFBP-3, and testosterone
- define how diet and BMI impact serum levels of IGF-1, IGFBP-2, IGFBP-3, testosterone and PSA in African American and Caucasian men.
- determine the associations between serum levels of free and total IGF-1, IGFBP-2, IGFBP-3, testosterone, PSA, BMI and specific nutrients.

The proposed study will help to explain the increased risk of prostate cancer for African American men and the role of specific nutrients in influencing IGF-1 and IGF-binding protein concentrations.

BODY

Study progress during the first and second year of funding will be described below, with respect to each of the tasks listed in the Statement of Work.

Statement of Work

Task 1: Months 1-3: Hiring and Training of Staff
The grant was officially awarded December 1999, but did not start until April 2000 due to concerns expressed by the Human Subjects Protection, AMDEX Corporation. In March, a medical research assistant was employed to work on the project. Study protocol was finalized and a training session was held to discuss study goals, objectives, protocols, responsibilities and data collection procedures.

Task 2: Months 3-4: Obtain and review clinical questionnaires of 1,517 men who participated in prostate screenings to identify men eligible for the study
The clinical questionnaires were obtained from the men who participated in the prostate screenings. The questionnaires were categorized by race, age, and cancer status. Computer entries of all questionnaires were inputted in Microsoft Excel.

Task 3: Months 4-5: Obtain PSA values for men who are eligible for the study.
PSA results were obtained for all men who had stored serum. Computer entry of results was inputted in Microsoft Excel.

Task 4: Months 4-5: Work with Director of Serum Bank to retrieve serum for men eligible for the study.

We are worked closely with the Dr. Bruce Trock, who was the Serum Bank Director, Lombardi Cancer Center, Georgetown University. Dr. Trock informed us that many of the stored samples were frozen in the wrong tubes, stored as whole blood, or were not centrifuged. Therefore, we conducted preliminary analysis to determine the reliability and validity of IGF-1, IGFBP-2, and IGFBP-3 in whole blood when compared to serum. Samples were obtained from 10 volunteers participating in Dr. Trocks project. Dr. Kevin Cullen, who is an investigator with this project, had his lab to conduct the comparative analysis. Results from the analysis revealed that the samples were not appropriate for our study. Therefore, we recruited new men who came to prostate

screenings at the Lombardi Cancer Center and the Howard University Cancer Center. To date, we have recruited 544 men and have collected serum samples for this project.

Task 5: Months 5-8: Analyze serum for IGF-1, IGFBP-2, IGFBP-3 and testosterone.

Serum analysis is currently being conducted in Dr. Kevin Cullen's laboratory at the Lombardi Cancer Center. To date, approximately 599 assays are completed. This includes 271 assays analyzed for IGF-1, 200 assays analyzed for IGFBP-2 and 128 assays analyzed for IGFBP-3. *See appendices for assay methodology, assays completed and standard curves.*

Task 6: Months 6: Stratify and randomize over 300 men for telephone interview.
We have stratified and randomized approximately 100 men who are eligible for the telephone interview.

Task 7: Months 6-8: Send letters to 300 men requesting telephone interview.
Letters have been sent to 100 men requesting an interview.

Task 8: Months 7-13: Call 300 men to schedule telephone interview.
Approximately 40 men have scheduled an interview.

Task 9: Months 8-20: Conduct telephone interview.
Twenty- five men were administered a nutrition food frequency questionnaire over the telephone.

Task 10: Months 9-21: Mail monetary incentive to interviewees.
Monetary incentives have been sent to 20 men who completed the interview.

Task 11: Months 15-24: Data entry and analyze; complete final report.
Have not yet addressed.

KEY RESEARCH ACCOMPLISHMENTS

- Hired and trained personnel working on project.
- Finalization of study protocol.
- Obtained and reviewed clinical questionnaires of 1,517 men who had stored serum.
- Preliminary analysis to determine if stored blood was appropriated for our study.
- Obtained PSA values for men who had stored blood.
- Data entry of clinical information from questionnaires and PSA values.
- Recruited 544 men who participated in recent prostate screenings.
- Completed 599 assays consisting of IGF-1, IGFBP-2, IGFBP-3.
- Stratified and randomized 100 men for the telephone interview.
- Conducted telephone interview with 25 study participants.

REPORTABLE OUTCOMES
None at this time.

CONCLUSIONS

Study personnel was hired and trained. The clinical protocol was finalized. Approximately 1500 clinical questionnaires were reviewed to determine which men were eligible for the study. Data entry of clinical information and PSA's were completed for all eligible subjects. However, there were unanticipated obstacles in sorting out which frozen blood samples were appropriate for analysis of study variables. Preliminary analysis was conducted to compare the validity and reliability of IGF-1, IGFBP-2, and IGFBP-3 in whole blood versus serum. It was determined that the frozen blood samples (whole blood) were not appropriate for use in this study.

Since the frozen samples of whole blood could not be used for this project, we began recruiting men who attended prostate screenings at the Lombardi Cancer Center and the Howard University Cancer Center. To date, we have recruited 544 men. From these men, approximately 599 assays have been analyzed for IGF-1, IGFBP-2, and IGFBP-3. A total of 100 men were stratified and randomized for the telephone interview. Letters requesting a telephone interview were sent to 100 men. Of this number 25 men were interviewed to determine nutrition intake.

In December 2001, we requested a no-cost one-year extension of this project to allow additional time for recruiting study participants and to analyze serum samples for study variables. Our request was approved. We are confident that the study objectives will be completed at that time.

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APPENDICES

LIST OF ABBREVIATIONS AND ACRONYMS

IGF   insulin growth factor type 1
IGFBP-2   insulin growth factor binding protein 2
IGFBP-3   insulin growth factor binding protein 3
PSA   prostate-specific androgen

SERUM ASSAY METHODOLOGY

IGFBP-2 Assay

IGFBP2 Radioimmunoassay (RIA) (Diagnostics Systems Laboratories (DSL), Webster, Texas; kit DSL-7100): Serum samples were assayed in duplicate according to the manufacturer's instructions. The RIA procedure measures competition between a radioactive and non-radioactive antigen for a fixed number of antigen binding sites. The amount of I125-labeled IGFBP-2 bound to the antibody is inversely proportional to the concentration of unlabeled IGFBP-2 present. Separation of the free and bound antigen is achieved by using a double antibody system. Briefly, serum specimens were pre-incubated with anti-IGFBP2 polyclonal antibody, incubated further (overnight) after the addition of I125-labeled IGFBP-2, and antigen-bound antibody was precipitated using polyclonal anti-rabbit gamma globulin serum in a buffer containing polyethylene glycol. Sample radioactivity was measured in a gamma counter (Packard Cobra II Auto-Gamma). Results were determined from a semilog standard curve plotting %B/Bzero [mean sample counts – nonspecific background counts (NSB)] / [mean counts of 0 ng/ml standard – NSB] versus the log of standard IGFBP-2 concentrations, as recommended by the manufacturer.

Two supplied controls were included on each assay plate, Level I (low, 5.5 +/- 1.6) and Level II (high, 18 +/- 5.4). On one occasion (12/14/01 assay), the Level II assayed value (24. 8) was slightly outside the confidence interval determined by the manufacturer (12.6 - 23.4). The Level I value was within range (6.1). The modest departure of the Level II value from the confidence interval was not considered sufficient to exclude the assay results. For all other assays, control values fell within range.

Serum samples were diluted 1:30, 1:40 or 1:50. The first assay (11/21/01) was performed using the manufacturer's typical recommended dilution of 1:50. Based on those results, where 11 of 40 samples fell below the lowest standard, the dilution was adjusted to 1:40 for the second assay (12/1/01). Since 10 of 40 samples fell below the lowest standard at that dilution, the dilution was adjusted to 1:30 for the third assay (12/7/01), and all but one sample fell within range of the standard curve. Subsequent assays were performed using a dilution of 1:30. Samples for which a serum IGFBP-2 value could not determine because the diluted sample was below the lowest standard will be retested at a lower dilution. All other values within the range of the standard curve were valid and are reported.

Serum IGF1 Assay

Non-extraction IGF-1 Enzyme-Linked Immunosorbent Assay (ELISA) (DSL, kit DSL-10-2800): Serum samples were assayed in duplicate according to the manufacturer's instructions. The ELISA procedure is an enzymatically amplified sandwich immunoassay. Absorbance measurement from a colorimetric reaction is directly proportional to the concentration of IGF-1 present. Briefly, following overnight

pretreatment in sample buffer, samples were incubated in microplate wells coated with an anti-IGF1 antibody. Wells were washed, and enzyme-conjugated anti-IGF1 antibody added for a second incubation. Following washing, the substrate tetramethylbenzidine was added. The reaction was stopped after ten minutes with an acidic stopping solution, and the absorbance at 450 nm determined using a microplate reader (Molecular Devices THERMOmax). Sample IGF1 values were determined from a standard curve plotting the log of mean absorbance versus the log of standard IGF-1 concentrations, as recommended by the manufacturer.

Two supplied controls were included on each assay plate. For all assays, assayed values for the controls were within the manufacturer's confidence intervals.

Serum IGFBP-3 Assay

IGFBP-3 ELISA (DSL, kit DSL-10-6600): Serum samples were assayed in duplicate according to the manufacturer's instructions. Briefly, diluted serum samples were incubated in microplate wells coated with an anti-IGFBP-3 polyclonal antibody. Wells were washed, and enzyme-conjugated anti-IGFBP-3 polyclonal antibody added for a second incubation. Following washing, the substrate tetramethylbenzidine was added. The reaction was stopped after ten minutes with an acidic stopping solution, and the absorbance at 450 nm determined using a microplate reader. Sample IGFBP-3 values were determined from a standard curve plotting the log of mean absorbance versus the log of standard IGFBP-3 concentrations, as recommended by the manufacturer.
Two supplied controls were included on each assay plate. For each assay, assayed values for the controls were within the confidence interval determined by the manufacturer.

		IGF-BP3	
sample	Patient	ng/ml	SEM
1	628	4183	31
2	624	3682	29
3	735	3967	73
4	761	1935	4
5	504	3861	51
6	3	4452	97
7	782	3024	32
8	795	2221	137
9	28	2220	74
10	800	3022	172
11	32	3470	94
12	750	3548	61
13	584	2876	45
14	33	2287	25
15	31	4878	404
16	788	2738	40
17	654	3483	11
18	745	2341	110
19	10	2635	354
20	34	4342	157
21	41	3518	2
22	4	2782	19
23	14	2778	0
24	535	3981	420
25	451	2632	70
26	611	2977	34
27	476	3478	120
28	467	3739	93
29	657	3159	35
30	38	3179	108
31	609	3752	26
32	36	3942	40
33	69	2627	219
34	792	3154	532
35	16	2600	15
36	748	3250	86
37	471	3050	92
38	525	2395	63
39	614	4717	68
40	105	3958	71

Number of values	40
Minimum	1935
25% Percentile	2687
Median	3169
75% Percentile	3807
Maximum	4878
Mean	3271
Std. Deviation	728.2
Std. Error	115.1
Lower 95% CI	3038
Upper 95% CI	3504

Controls	observed	expected
Level I	5.7 +/- .2	4.5 +/- 1.5
Level II	22.7 +/- .6	18 +/- 5.5

1/31/02 IGFBP-3 ELISA Standard Curve
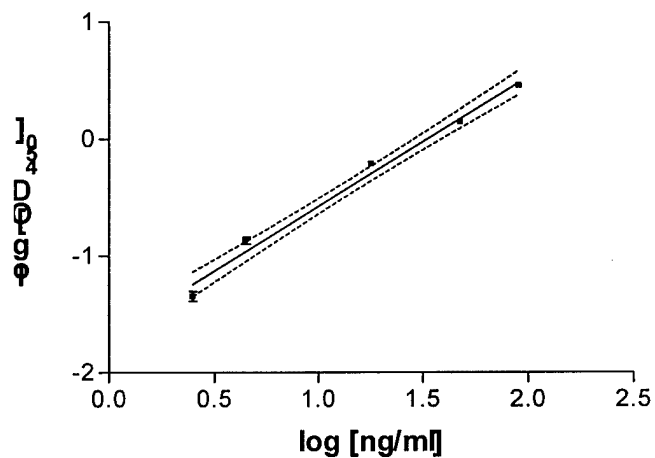
sample	Patient	IGF-BP3 ng/ml	SEM
1	115	2549	12
2	29	2082	44
3	640	3514	133
4	63	3131	1109
5	92	3464	290
6	528	2219	76
7	70	2692	203
8	612	3452	319
9	15	2975	356
10	615	2288	233
11	758	2807	261
12	602	2843	37
13	770	3084	8
14	581	2404	125
15	656	3241	58
16	769	2822	65
17	72	2368	137
18	626	2656	232
19	565	2712	29
20	618	2436	93
21	540	2384	137
22	23	2018	44
23	762	2816	384
24	90	2667	41
25	491	1528	98
26	73	2659	57
27	508	2091	188
28	690	2509	69
29	747	2598	12
30	51	3431	41
31	520	2082	4
32	727	1599	43
33	548	2986	98
34	821	2505	105
35	732	2295	72
36	485	2639	28
37	145	3031	185
38	822	4488	21
39	590	3859	79
40	513	4686	0

Number of values	40
Minimum	1528
25% Percentile	2376
Median	2663
75% Percentile	3058
Maximum	4686
Mean	2765
Std. Deviation	657.2
Std. Error	103.9
Lower 95% CI	2555
Upper 95% CI	2975

Controls	observed	expected
Level I	5.1 +/- 0.0	4.5 +/- 1.5
Level II	21.6 +/- 1.0	18 +/- 5.5

2/7/02 IGFBP-3 ELISA Standard Curve
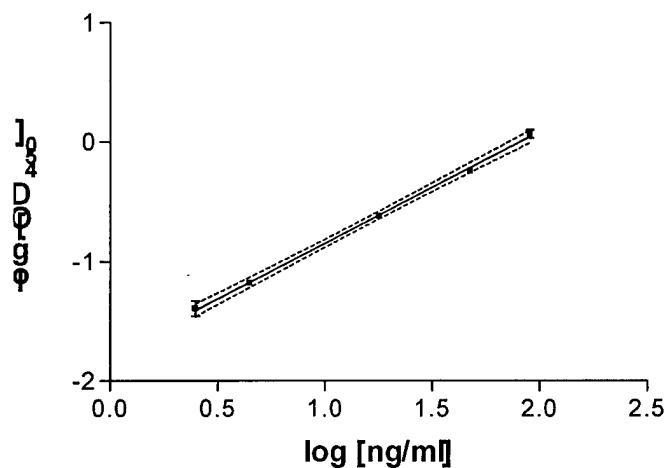
PLATE 1
IGFBP-3

Sample	Patient	ng/ml	SEM
1	5	3155	14
2	9	4806	82
3	22	3590	10
4	29	4100	46
5	31	3645	12
6	32	3272	38
7	33	2650	175
8	34	2827	14
9	35	3707	54
10	36	5447	143
11	37	3151	22
12	38	4704	40
13	39	4449	40
14	40	3292	79
15	41	4734	261
16	42	5094	40
17	43	3322	194
18	46	5737	168
19	48	3306	105
20	49	3504	225
21	50	3023	12
22	51	3250	174
23	52	6307	0
24	53	3733	16
25	55	3779	42
26	56	3433	75
27	58	4401	4
28	59	3163	42
29	60	3250	36
30	61	5272	28
31	62	5357	248
32	63	5816	160
33	64	3564	109
34	65	4941	157
35	66	3721	36
36	67	3540	64
37	68	3520	80
38	70	4381	279
39	71	3290	56
40	72	3280	14

Number of values	40
Minimum	2650
25% Percentile	3285
Median	3617
75% Percentile	4719
Maximum	6307
Mean	3988
Std. Deviation	929
Std. Error	146.9
Lower 95% CI	3691
Upper 95% CI	4285

Controls	observed	expected
Level I	4.4 +/- 0.0	4.5 +/- 1.5
Level II	15.0 +/- 0.4	18 +/- 5.5

4/16/01 IGFBP-3 ELISA
Standard Curve
Plate 1
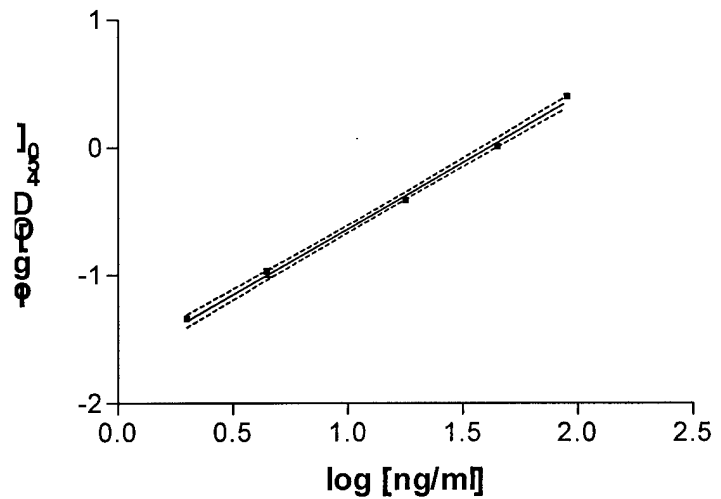
PLATE 2
IGFBP-3

Sample	Patient	ng/ml	SEM
1	73	6290	105
2	75	3577	132
3	77	4465	121
4	78	6746	343
5	79	3114	78
6	80	3504	45
7	82	4316	25
8	84	4660	43
9	85	3214	3
10	88	4114	39
11	89	3702	27
12	89	3343	107
13	91	5059	175
14	92	3418	32
15	93	2936	24
16	94	4086	34
17	95	3696	164
18	96	5768	260
19	99	3658	104
20	100	2615	57
21	101	4466	86
22	102	4093	52
23	103	4078	243
24	104	4278	88
25	105	5657	183
26	106	5423	179
27	107	3100	18
28	108	4815	257
29	109	4910	279
30	110	4239	186
31	111	4329	156
32	112	2709	17
33	113	5206	22
34	114	2538	148
35	115	3114	66
36	116	3766	85
37	117	5315	4
38	118	3919	101
39	119	4151	224
40	120	4847	114.0

Number of values	40
Minimum	2538
25% Percentile	3461
Median	4104
75% Percentile	4831
Maximum	6746
Mean	4181
Std. Deviation	994.4
Std. Error	157.2
Lower 95% CI	3863
Upper 95% CI	4499

Controls	observed	expected
Level I	5.1 +/- 0.0	4.5 +/- 1.5
Level II	17.1 +/- 0.1	18 +/- 5.5

4/16/01 IGFBP-3 ELISA Standard Curve Plate 2
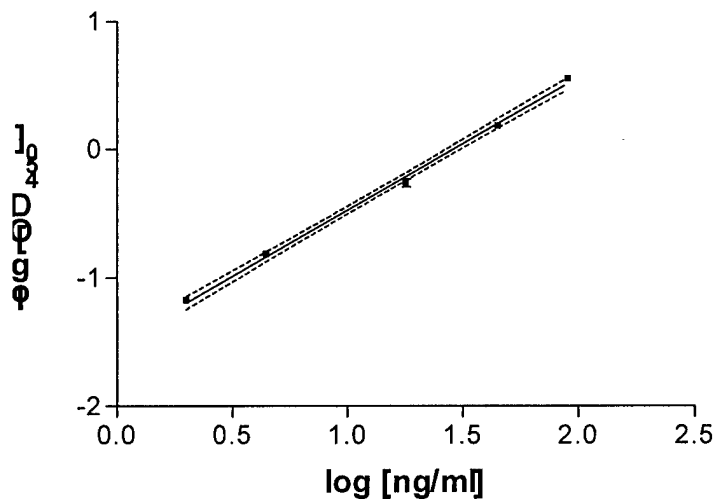
PLATE 3
IGFBP-3

Sample	Patient	ng/ml	SEM
1	121	4523	13.9
2	122	5809	64.1
3	123	5115	119.6
4	124	3355	62.3
5	125	5046	44.5
6	126	3574	29.5
7	127	4455	168.0
8	128	6120	332.0
9	129	4418	146.4
10	130	2251	28.4
11	131	2707	204.0
12	132	3349	281.8
13	133	4705	81.5
14	134	2211	36.4
15	135	4898	483.7
16	136	3667	228.1
17	137	5162	118.0
18	139	4051	208.7
19	140	4498	417.4
20	141	5710	236.7
21	142	6614	4.6
22	144	6394	215.9
23	145	2631	4.7
24	146	4255	103.4
25	147	3681	31.0
26	148	6081	574.3
27	149	2309	11.1
28	150	4998	46.0
29	151	6216	7.6
30	151	6548	53.2
31	152	4198	24.7
32	153	5220	191.5
33	155	4574	36.9
34	156	5818	149.5
35	156	5493	209.5
36	157	3252	202.6
37	159	2952	81.3
38	160	3695	79.1
39	161	5395	53.6
40	162	5193	133.3

Number of values	40
Minimum	2211
25% Percentile	3620
Median	4549
75% Percentile	5444
Maximum	6614
Mean	4529
Std. Deviation	1253
Std. Error	198
Lower 95% CI	4128
Upper 95% CI	4929

Controls	observed	expected
Level I	5.1 +/- 0	4.5 +/- 1.5
Level II	17.8 +/- .5	18 +/- 5.5

4/16/01 IGFBP-3 ELISA Standard Curve Plate 3
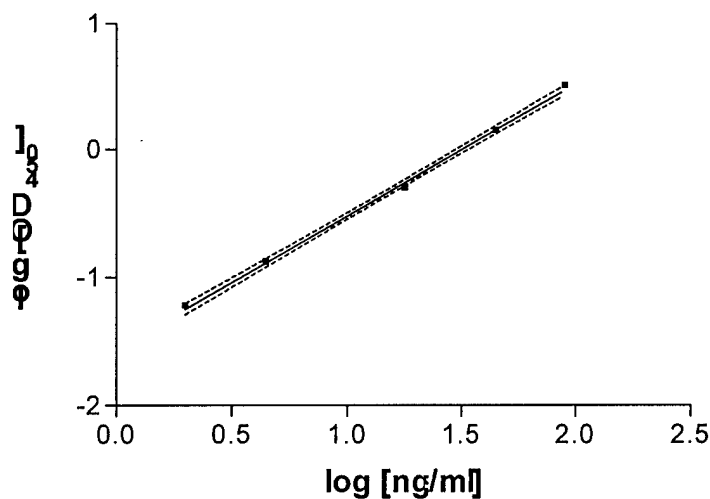
PLATE 4
IGFBP-3

Sample	Patient	ng/ml	SEM
1	163	4486	4395.3
2	164	4249	4011.2
3	165	3760	3596.4
4	166	4249	4140.4
5	167	5965	6513.3
6	168	4409	4465.2
7	169	3576	3662.6
8	170	5141	5670.1
9	172	4172	4084.5
10	173	5239	5522.7
11	174	4906	5252.8
12	175	5218	5371.9
13	176	4151	4594.6
14	177	5347	5596.4
15	178	4325	4559.6
16	182	4630	5179.2
17	183	5291	5347.4
18	184	2153	2163.6
19	185	4486	4671.5
20	186	3057	3217.1
21	Crump	5712	5943.9
22	Darnell	5015	5210.7
23	Davis	3210	3669.6
24	Franko	4619	4941.0
25	Gerry	2651	2738.0
26	Harris	4616	4962.0
27	Key	3516	4091.5
28	Rogers	5193	5378.9

Number of values	28
Minimum	2153
25% Percentile	3956
Median	4486
75% Percentile	5167
Maximum	5965
Mean	4405
Std. Deviation	914.2
Std. Error	172.8
Lower 95% CI	4051
Upper 95% CI	4760

Controls	observed	expected
Level I	4.2 +/- 0.1	4.5 +/- 1.5
Level II	15.5 +/- 0.0	18 +/- 5.5

4/16/01 IGFBP-3 ELISA
Standard Curve
Plate 4
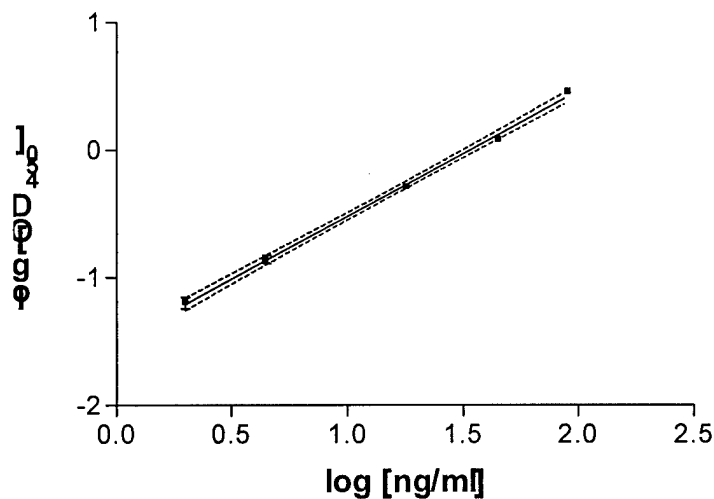
sample	patient	IGF1 ng/ml	SEM
1	628	155	3.6
2	624	178	1.9
3	735	164	2.0
4	761	85	0.7
5	504	133	0.7
6	3	169	0.5
7	782	105	0.5
8	795	52	0.4
9	28	99	0.4
10	800	79	1.4
11	32	153	0.0
12	750	130	2.0
13	584	84	2.7
14	33	66	0.0
15	31	224	1.6
16	788	129	1.9
17	654	107	0.1
18	745	72	0.6
19	10	74	1.7
20	34	162	0.1
21	41	173	0.3
22	4	153	0.9
23	14	107	0.0
24	535	170	2.2
25	451	146	1.1
26	611	153	1.8
27	476	103	2.7
28	467	155	3.9
29	657	133	0.3
30	38	172	1.1
31	609	204	1.5
32	36	219	3.6
33	69	166	2.0
34	792	134	2.3
35	16	131	3.5
36	748	139	2.7
37	471	92	2.2
38	525	161	3.9
39	614	232	3.9
40	105	133	3.0
41	91	137	1.5

Stats	
Number of values	41
Minimum	52.45
25% Percentile	105.5
Median	137.1
75% Percentile	162.2
Maximum	232.3
Mean	137.4
Std. Deviation	43.47
Std. Error	6.789
Lower 95% CI	123.7
Upper 95% CI	151.1

control	observed	expected
Level I	101 +/- 6.7	100 +/- 25
Level II	336 +/- 13.9	300 +/- 75

2/1/02 IGF1 ELISA Standard Curve

log [ng/ml IGF1]
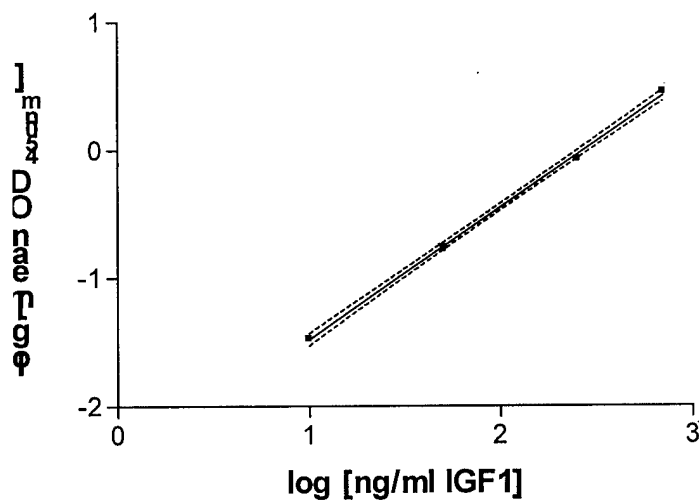
sample	patient	IGF1 ng/ml	SEM
1	115	75	1.5
2	29	65	0.9
3	640	139	0.2
4	63	131	0.8
5	92	228	1.3
6	528	116	1.2
7	70	134	0.8
8	612	229	0.8
9	15	109	2.6
10	615	73	0.3
11	758	145	7.5
12	602	189	2.1
13	770	197	1.0
14	581	113	1.2
15	656	111	2.2
16	769	138	0.2
17	72	71	1.0
18	626	71	4.9
19	565	130	2.2
20	618	83	2.4
21	540	122	0.5
22	23	120	0.6
23	762	185	3.1
24	90	149	0.0
25	491	74	2.4
26	73	85	3.3
27	508	41	2.1
28	690	90	2.6
29	747	114	6.1
30	51	151	2.4
31	520	125	0.9
32	727	145	2.1
33	548	120	1.7
34	821	56	1.9
35	732	87	14.3
36	485	71	1.8
37	145	125	13.8
38	822	141	1.1
39	590	137	2.1
40	513	188	3.9
41	559	152	5.4

Stats	
Number of values	41
Minimum	40.55
25% Percentile	84.61
Median	121.6
75% Percentile	140.8
Maximum	229.2
Mean	122.5
Std. Deviation	44.87
Std. Error	7.007
Lower 95% CI	108.3
Upper 95% CI	136.7

control	observed	expected
Level I	83 +/- .3	100 +/- 25
Level II	296 +/- 13	300 +/- 75

2/8/02 IGF1 ELISA Standard Curve

log [ng/ml IGF1]
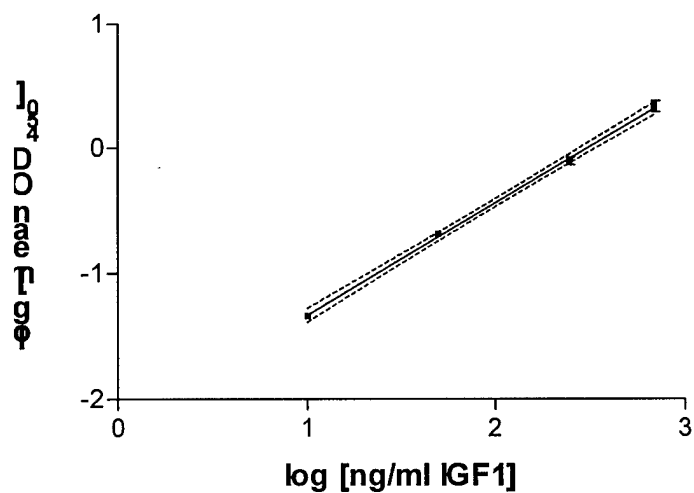
sample	patient	IGF1 ng/ml	SEM
1	541	93	0.0
2	482	234	3.8
3	607	183	1.2
4	149	122	1.6
5	728	165	1.6
6	473	128	0.6
7	671	93	0.1
8	462	130	0.6
9	131	115	0.7
10	465	75	0.4
11	823	126	0.1
12	486	131	0.4
13	494	129	0.3
14	642	101	0.3
15	150	137	0.3
16	603	151	0.9
17	689	180	2.7
18	83	95	0.9
19	477	109	0.3
20	464	178	0.2
21	469	94	0.6
22	736	214	2.0
23	152	180	0.5
24	459	144	2.1
25	556	133	0.4
26	551	134	0.6
27	480	187	0.8
28	644	106	0.6
29	88	108	1.6
30	460	215	3.8
31	596	127	0.4
32	52	192	1.2
33	583	176	0.3
34	42	121	0.3
35	85	147	3.0
36	759	217	0.9
37	601	123	3.1
38	678	156	2.4
39	552	189	3.6
40	700	68	2.1
41	559	114	0.9

Stats	
Number of values	41
Minimum	67.51
25% Percentile	113.5
Median	130.8
75% Percentile	175.7
Maximum	233.6
Mean	141.9
Std. Deviation	41.13
Std. Error	6.424
Lower 95% CI	128.9
Upper 95% CI	154.9

control	observed	expected
Level I	106 +/- 1.0	100 +/- 25
Level II	345 +/- 5.2	300 +/- 75

3/1/02 IGF1 ELISA Standard Curve

log [ng/ml IGF1]
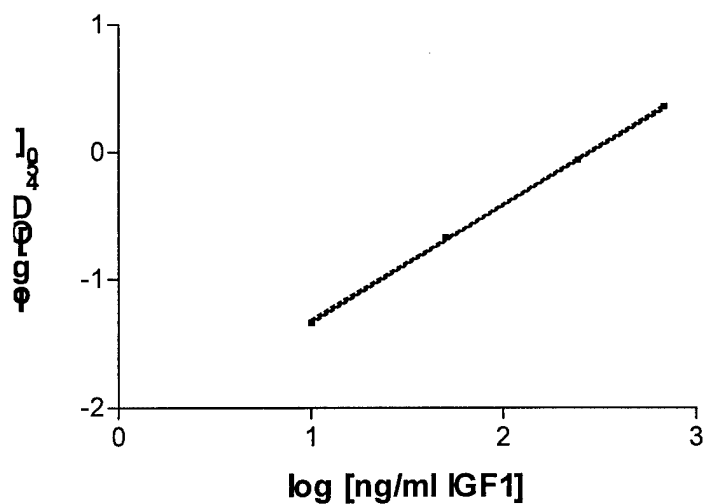
PLATE 1

Sample	Patient	IGF1 ng/ml	SEM
1	5	142	0.6
2	9	184	1.1
3	22	160	1.4
4	29	159	0.9
5	31	157	3.0
6	32	120	4.8
7	33	137	5.4
8	34	85	2.5
9	35	71	1.5
10	36	133	1.8
11	37	100	2.6
12	38	206	3.2
13	39	199	4.5
14	40	131	5.9
15	41	190	1.6
16	42	188	0.1
17	43	185	3.9
18	46	137	1.7
19	48	117	3.4
20	49	136	4.8
21	50	110	0.8
22	51	123	1.1
23	52	202	0.6
24	53	116	0.7
25	55	136	2.9
26	56	96	0.8
27	58	132	1.7
28	59	122	0.7
29	60	87	0.9
30	61	134	1.9
31	62	124	2.9
32	63	140	0.3
33	64	86	0.1
34	65	139	1.5
35	66	116	0.6
36	67	128	0.5
37	68	132	1.9
38	70	179	0.3
39	71	115	3.3
40	72	120	0.4
41	73	295	2.0

Number of values	41
Minimum	71.44
25% Percentile	116.6
Median	132.9
75% Percentile	156.9
Maximum	295.3
Mean	140.7
Std. Deviation	41.67
Std. Error	6.507
Lower 95% CI	127.5
Upper 95% CI	153.8

Controls	observed	expected
Level I	59 +/- 2.2	55 +/- 15
Level II	202 +/- .4	215 +/- 60

4/16/01 IGF1 ELISA
Standard Curve
Plate 1

log [mg/ml IGF1]
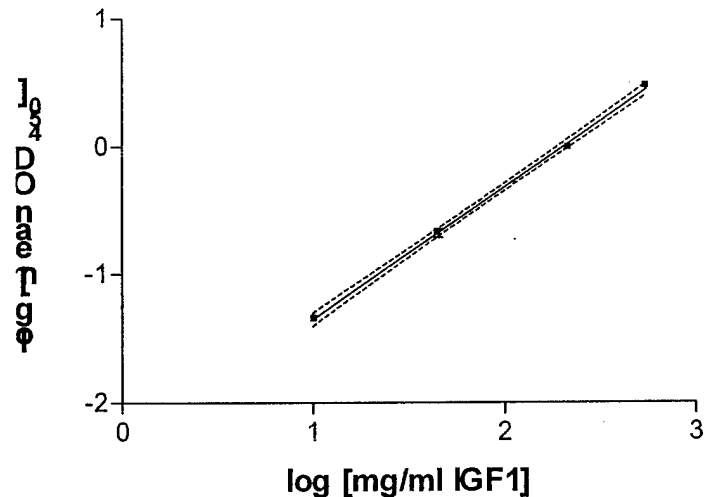
PLATE 2

Sample	Patient	IGF1 ng/ml	SEM
1	75	178	3.6
2	77	146	1.0
3	78	148	2.1
4	79	146	0.3
5	80	130	0.3
6	82	185	1.2
7	84	156	0.6
8	85	89	4.4
9	88	218	2.2
10	89	111	4.5
11	89	104	4.5
12	91	128	7.6
13	92	176	11.1
14	93	134	6.1
15	94	136	4.2
16	95	103	1.0
17	96	239	16.0
18	99	241	8.7
19	100	123	3.8
20	101	146	10.2
21	102	157	10.1
22	103	103	7.3
23	104	160	0.8
24	105	119	7.6
25	106	242	14.3
26	107	91	2.3
27	108	190	10.6
28	109	171	5.0
29	110	144	0.9
30	111	86	7.2
31	112	99	3.2
32	113	210	10.3
33	114	137	7.6
34	115	144	7.4
35	116	135	10.1
36	117	135	3.8
37	118	139	17.4
38	119	139	12.3
39	120	210	14.6
40	121	188	10.4
41	122	206	2.0

Number of values	41
Minimum	85.83
25% Percentile	128.3
Median	144.2
75% Percentile	176.4
Maximum	241.6
Mean	152.3
Std. Deviation	42.35
Std. Error	6.614
Lower 95% CI	138.9
Upper 95% CI	165.6

Controls	observed	expected
Level I	70.4 +/- 2.0	55 +/- 15
Level II	210 +/- 3.1	215 +/- 60

4/16/01 IGF1 ELISA Standard Curve Plate 2

log [mg/ml IGF1]
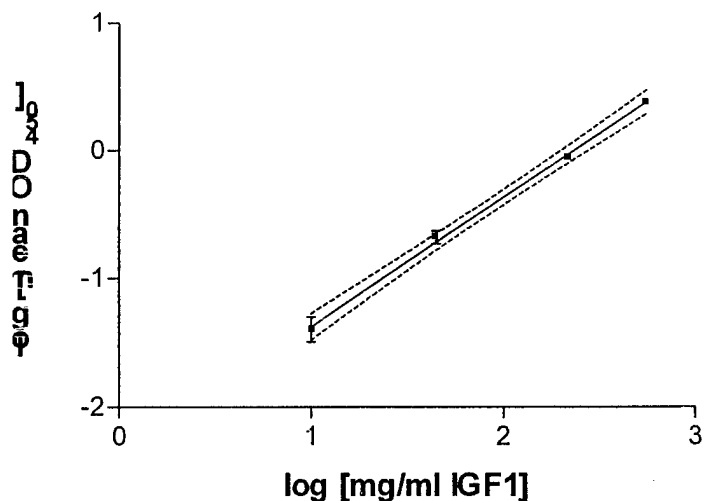
PLATE 3

Sample	Patient	IGF1 ng/ml	SEM
1	123	163	5.4
2	124	123	5.4
3	125	129	3.9
4	126	87	2.1
5	127	134	2.8
6	128	117	4.0
7	129	152	5.3
8	130	51	1.5
9	131	88	2.0
10	132	121	4.9
11	133	109	10.0
12	134	52	4.9
13	135	113	1.5
14	136	104	2.0
15	137	185	4.3
16	139	112	9.0
17	140	123	5.4
18	141	183	6.1
19	142	162	2.8
20	144	146	3.4
21	145	63	6.0
22	146	118	1.8
23	147	100	3.9
24	148	121	9.8
25	149	37	0.1
26	150	136	3.5
27	151	197	11.7
28	151	182	6.0
29	152	168	9.9
30	153	143	6.4
31	155	129	7.9
32	156	123	1.5
33	156	135	0.9
34	157	138	1.9
35	159	107	9.7
36	160	92	7.7
37	161	168	7.5
38	162	115	5.5
39	163	141	14.6
40	164	92	7.6
41	165	174	4.4

Number of values	41
Minimum	36.55
25% Percentile	106.8
Median	122.9
75% Percentile	143.5
Maximum	197
Mean	125.2
Std. Deviation	37.5
Std. Error	5.856
Lower 95% CI	113.4
Upper 95% CI	137

Controls	observed	expected
Level I	69 +/- 1.0	55 +/- 15
Level II	206 +/- 4.2	215 +/- 60

4/16/01 IGF1 ELISA Standard Curve Plate 3

log [mg/ml IGF1]
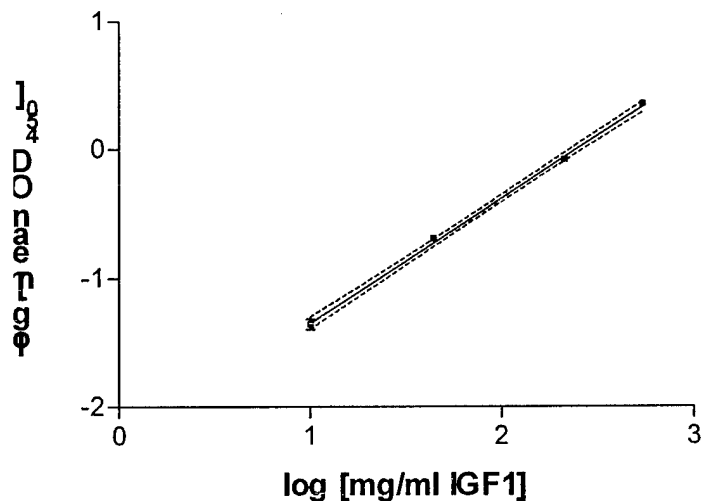
PLATE 4

Sample	Patient	IGF1 ng/ml	SEM
1	166	127	3.9
2	167	260	3.5
3	168	162	3.1
4	169	112	7.3
5	170	146	0.3
6	172	185	10.0
7	173	135	4.1
8	174	133	4.2
9	175	161	3.3
10	176	146	2.3
11	177	179	5.3
12	178	.170	11.0
13	182	106	6.1
14	183	169	1.6
15	184	91	1.4
16	185	174	1.5
17	186	142	5.4
18	Crump	215	2.6
19	Darnell	133	2.8
20	Davis	115	3.4
21	Franko	164	5.6
22	Gerry	82	0.5
23	Harris	208	1.1
24	Key	116	4.4
25	Rogers	197	2.8

Number of values	25
Minimum	81.9
25% Percentile	127
Median	145.7
75% Percentile	170.5
Maximum	260.2
Mean	153
Std. Deviation	41.27
Std. Error	8.254
Lower 95% CI	136
Upper 95% CI	170

Controls	observed	expected
Level I	68 +/- .4	55 +/- 15
Level II	209 +/- 4.5	215 +/- 60

4/16/01 IGF1 ELISA
Standard Curve
Plate 4

IGFBP-2
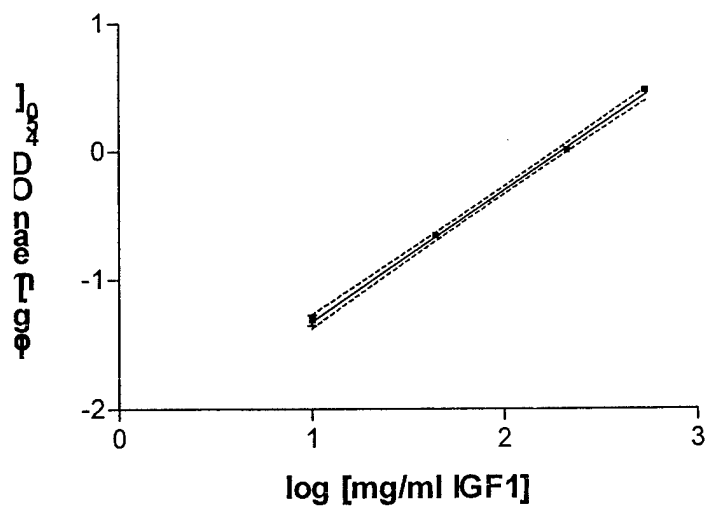
Assay Date:		11/21/01			12/1/2001	
sample dilution:		1:50			1:40	
	Sample No.	Pt No.	ng/ml	Sample No.	Pt No.	ng/ml
	1	561	345.8	1	689	278.6
	2	496	447.0	2	462	
	3	472		3	83	284.7
	4	165	383.1	4	131	529.4
	5	163	1191.9	5	477	231.5
	6	159	435.0	6	465	
	7	164	475.4	7	464	1980.4
	8	470		8	823	
	9	155	477.7	9	469	266.3
	10	148	443.1	10	486	1043.7
	11	492		11	736	296.7
	12	153		12	494	399.0
	13	82	304.8	13	152	771.8
	14	604	783.9	14	642	322.9
	15	824	690.7	15	459	
	16	558		16	150	150.5
	17	682	346.2	17	556	
	18	553	621.4	18	603	548.0
	19	484	572.7	19	551	621.5
	20	86	475.8	20	590	170.5
	21	623	330.5	21	727	
	22	560	818.5	22	513	244.2
	23	638	1113.0	23	559	264.1
	24	562	279.9	24	548	316.2
	25	42	143.2	25	541	
	26	84	287.5	26	821	
	27	583	410.0	27	482	627.8
	28	700	297.7	28	732	225.2
	29	552	787.4	29	607	228.6
	30	52	790.1	30	485	439.6
	31	678	349.9	31	149	1347.1
	32	596	652.1	32	145	597.7
	33	601	404.3	33	728	
	34	460		34	473	305.0
	35	759		35	822	
	36	88		36	101	1109.4
	37	85		37	59	401.7
	38	644		38	586	230.2
	39	480		39	757	306.1
	40	671	502.4	40	458	517.2
	control 1	<2.5				5.8
	control 2	20.3				16.7

control ranges:
control 1: 3.9 - 7.1
control 2: 12.6 - 23.4

IGFBP-2

| | 12/7/2001 | | | 2/14/2001 | |
| | 1:30 | | | 1:30 | |
Sample No.	Pt. No.	ng/ml	Sample No.	Pt. No.	ng/ml
1	636	370.3	1	502	832.3
2	106	518.9	2	107	617.0
3	685	462.6	3	64	543.5
4	478	381.2	4	74	506.4
5	112	585.5	5	500	1330.2
6	95	342.6	6	538	226.7
7	103	1723.4	7	47	528.1
8	708	179.0	8	78	2141.8
9	597	1777.9	9	616	244.9
10	668	359.9	10	79	828.5
11	598	410.3	11	65	328.4
12	67	283.4	12	789	268.2
13	516	249.3	13	791	343.1
14	755	733.0	14	544	810.3
15	456	200.8	15	705	698.9
16	99	377.9	16	56	581.9
17	55	374.6	17	794	462.3
18	756	406.5	18	767	304.2
19	691	298.2	19	772	274.4
20	60	1002.6	20	54	285.7
21	68	264.8	21	497	302.3
22	667	203.4	22	50	239.1
23	488	155.0	23	13	1245.3
24	71	81.8	24	505	548.1
25	765	160.9	25	557	266.6
26	701	87.8	26	729	908.9
27	746	280.1	27	726	1096.1
28	681	712.7	28	738	216.7
29	764	111.7	29	466	255.9
30	751	350.3	30	734	496.5
31	679	154.8	31	651	261.5
32	97	496.5	32	588	234.0
33	637	796.6	33	113	216.6
34	686	908.3	34	455	290.3
35	75		35	611	287.3
36	474	602.6	36	476	240.5
37	781	583.7	37	467	299.3
38	43	422.5	38	657	289.4
39	61	579.7	39	38	482.6
40	452	248.1	40	609	233.0
		7.2			6.1
		20.2			24.8

IGFBP-2

12/16/2001

Sample No.	Pt. No.	1:30 ng/ml
1	36	252.3
2	69	1431.1
3	792	303.4
4	16	253.7
5	748	729.8
6	471	386.0
7	525	307.9
8	614	77.8
9	105	209.5
10	91	207.6
11	115	
12	29	545.2
13	640	626.6
14	63	507.1
15	528	235.2
16	92	399.9
17	612	350.9
18	70	620.7
19	615	619.5
20	15	342.8
21	602	1013.1
22	758	284.2
23	581	315.9
24	770	399.4
25	769	577.4
26	656	365.9
27	626	527.8
28	72	1055.9
29	618	312.3
30	565	698.3
31	23	1002.8
32	540	446.5
33	90	345.4
34	762	421.6
35	628	98.0
36	624	537.3
37	735	286.5
38	761	294.3
39	504	218.3
40	3	766.5

6.0

20.0

IGFBP-2

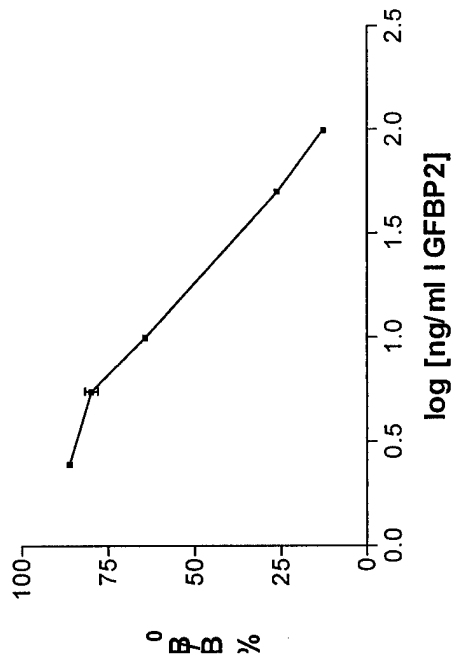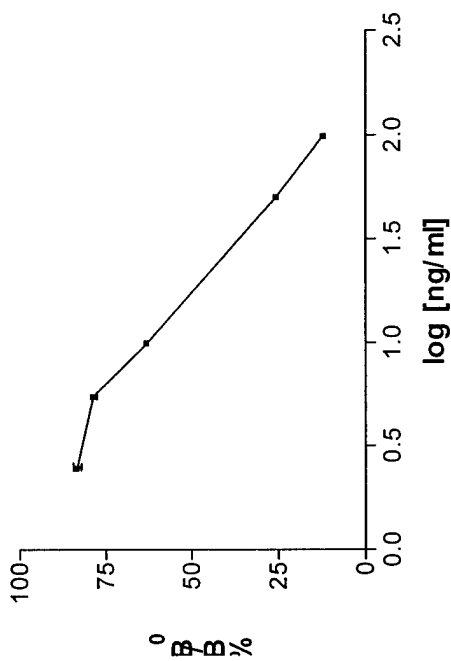| Assay date:	########	12/1/2001	12/7/2001	########	########
Stats					
Number of values	29	30	39	40	39
Minimum	143.2	150.5	81.8	216.6	77.84
25% Percentile	346.2	265.2	248.7	264	290.4
Median	447	319.5	374.6	316.3	386
75% Percentile	621.4	572.9	581.7	599.5	598.5
Maximum	1192	1980	1778	2142	1431
Mean	522.8	501.9	467.7	514.2	471.1
Std. Deviation	247.1	402.4	374.4	393.6	284.2
Std. Error	45.88	73.46	59.95	62.23	45.51
Lower 95% CI	428.8	351.6	346.3	388.3	379
Upper 95% CI	616.8	652.1	589	640	563.3

**11/21 IGFBP2 RIA
Standard Curve**

**12/1 IGFBP2 RIA
Standard Curve**

**12/7/01 IGFBP2 RIA
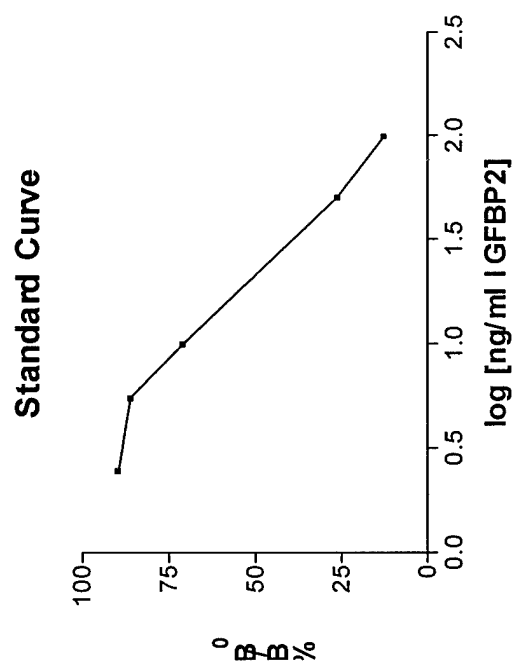Standard Curve**
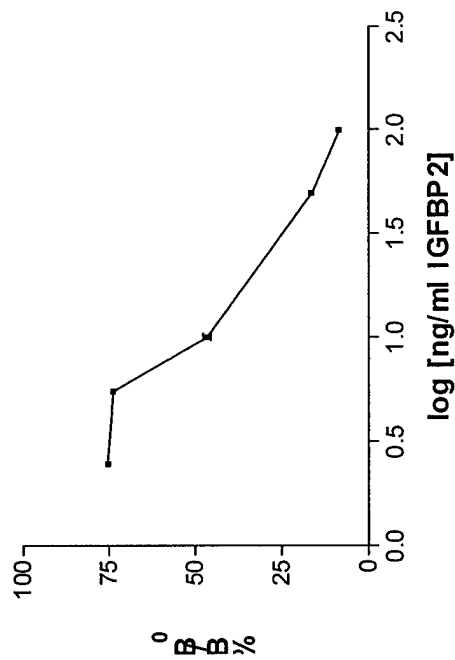
**12/14 IGFBP2 RIA
Standard Curve**
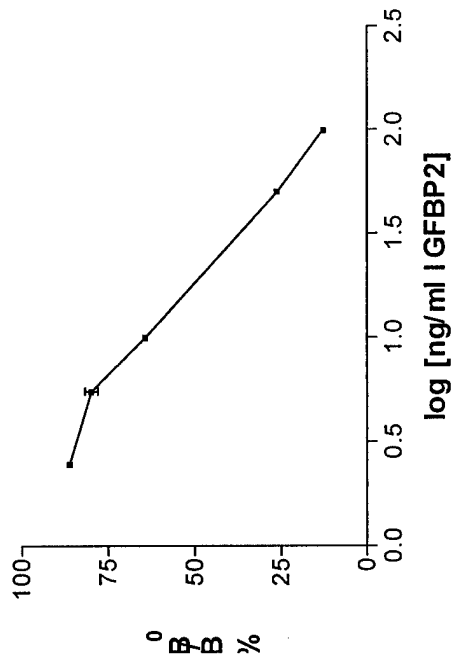
**12/16 IGFBP2 RIA
Standard Curve**

Standard Curve

Y-axis: % B/B₀ (100, 75, 50, 25, 0)

X-axis: log [ng/ml IGFBP2] (0.0, 0.5, 1.0, 1.5, 2.0, 2.5)
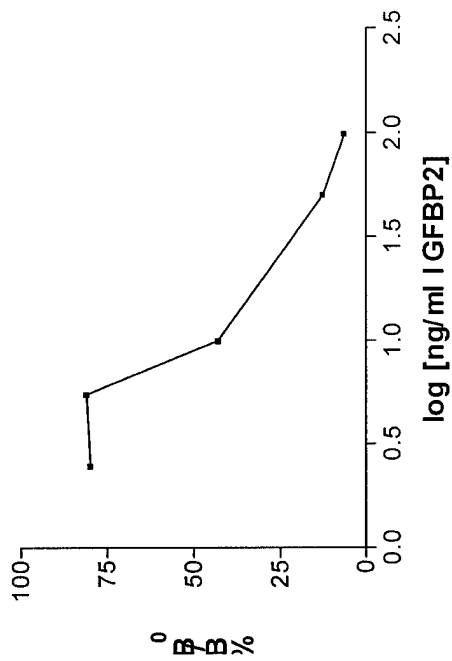
Meeting abstracts during reporting period: None in connection with this project

Publications during reporting period: None in connection with this project

Manuscripts in preparation: None in connection with this project

Personnel receiving pay from this negotiated effort (2001):

Everett Dodson
Kevin Cullen, M.D.
Tanya Agurs-Collins, Ph.D.